

REMARKS

The application as amended now complies with the requirements of 37 C.F.R. §§ 1.821-1.825 including:

- 1) a computer readable form (CRF) copy of the "sequence listing"
- 2) a paper copy of the "sequence listing" together with an amendment directing its entry into the specification and claims
- 3) a statement that the content of the paper and computer readable copies are the same and include no new matter

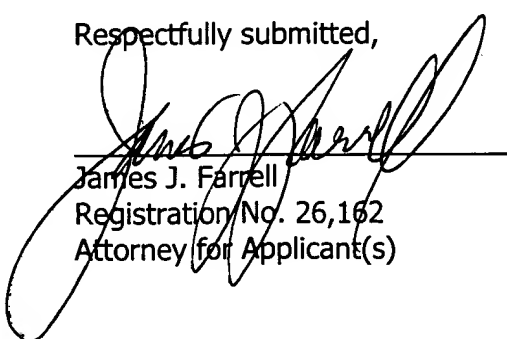
The claims as amended by the "annex" in the foreign prosecution, pages 39-42 inclusive (claims 1 to 16) have been cancelled.

Attached hereto is a marked-up version of the changes made to the specification and the claims by the current amendment. The attachment is captioned "Version With Markings To Show Changes Made".

Also attached hereto is a clean version of the entire set of pending claims. The attachments are captioned "Clean Version Of The Entire Set Of Pending Claims".

In view of the foregoing amendments, early favorable action is solicited.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In The Specification:

On page 3, please amend the paragraph beginning on line 17 and ending at the bottom of the page as follows:

Accordingly in a second aspect the invention relates to polypeptides comprising one or more fragments (A-E) having an amino acid sequence as follows:

SEQ ID NOS. 1-5, respectively, in order of appearance.

- (A) LEU-PRO-ASN-LEU-PHE-GLY-LYS
- (B) ILE-PRO-GLU-GLU-ILE-SER-ALA-LEU-LYS
- (C) LEU-THR-X-LEU-ASP-LEU-SER-PHE-ASN-LYS
- (D) SER-LEU-ARG-LEU-SER-SER-THR-SER-LEU-SER-GLY-PRO-VAL-PRO-LEU-PHE-PHE-PRO-GLN-LEU-X-LYS
- (E) X-X-GLU-VAL-ILE-PRO-X-GLN-LEU-SER-THR-LEU-PRO-ASN-LEU-LYS

On page 5, please amend the paragraph beginning on line 38 and ending at the bottom of the page as follows:

SEQ ID NOS. 6 & 7

Listing 1

Also embraced within the invention are isoforms and derivatives of the above mentioned polypeptides which still possess the antifreeze properties. Preferable the derivatives show at least 75% homology with the polypeptide of Listing 1 or the polypeptide comprising the partial sequences (A-E)

On page 17, please amend the paragraph beginning on line 1 and ending on line 16 as follows:

Several polypeptide fragments (A-E) were analyzed in the 36 kDa band and had sequences substantially homologous to:

SEQ ID NOS 1-5, respectively, in order of appearance

- (A) LEU-PRO-ASN-LEU-PHE-GLY-LYS
- (B) ILE-PRO-GLU-GLU-ILE-SER-ALA-LEU-LYS
- (C) LEU-THR-X-LEU-ASP-LEU-SER-PHE-ASN-LYS
- (D) SER-LEU-ARG-LEU-SER-SER-THR-SER-LEU-SER-GLY-PRO-VAL-PRO-LEU-PHE-PHE-PRO-GLN-LEU-X-LYS
- (E) X-X-GLY-VAL-ILE-PRO-X-GLN-LEU-SER-THR-LEU-PRO-ASN-LEU-LYS

On page 25, please amend the paragraph beginning on line 1 and ending on line 8 as follows:

Example VI

The peptide sequences shown in Example III were analyzed as to their suitability for degenerate oligonucleotide primer design. Part of Peptide D SEQ ID NO: 8 (GLY-PRO-VAL-PRO-LEU-PHE-PHE-PRO) was chosen and the primer cp3 SEQ ID NO:9 (GGI CCI GTI CCI YTI TTY TTY CC, where I=inosine and Y=C or T) was synthesized (Genosys).

On page 25, please amend the paragraph beginning on line 10 and ending at the bottom of the page as follows:

First strand cDNA was prepared from 5 µg cold acclimated (1 month as in example I) carrot root RNA using Superscript Reverse Transcriptase (Stratagene) and an oligonucleotide primer OG1 SEQ ID NO: 10 (GAGAGAGGATCCTCGAG(T)¹⁵) according to the manufacturer's instructions. 1% of the first strand cDNA reaction was used as template, together with cp3 and OG1 primers, in subsequent PCR. The reactions were carried out in a thermal cycler using Taq DNA polymerase (Gibco BRL) for 30 cycles (1 minute at 94 °C, 1 minute at 50 °C and 1 minute at 72 °C) according to the manufacturer's instructions. All primers were used at a concentration of 1 µM. The resulting ~800 bp PCR product was gel purified and cloned into the pTA_g vector (R&D Systems) according to the manufacturer's instructions. The cloned cp3 PCR product was sequenced using the dideoxy sequencing method employed by the Sequenase kit (USB). The cp3 nucleotide sequence and deduced amino acid sequence were substantially similar to:

SEQ ID NOS. 11 & 12

On page 27, please amend the paragraph beginning on line 24 and ending at the bottom of the page as follows:

Positive plaques were screened to purity and phage-mids excised before the inserts were characterised by DNA sequence analysis. Two cDNA clones were sequenced to completion. Although the 5' and 3' untranslated regions contained some sequence variability, the coding regions were identical. The coding regions of the two cDNA clones were substantially similar to:

SEQ ID NOS. 6 & 7